## Progress Report to the Southern Region Small Fruit Consortium

**Title:** Cross-pathogenicity to winegrape of *Xylella fastidiosa* cultures isolated from native vegetation

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**Objectives:** To determine the cross-pathogenicity of *Xylella fastidiosa* cultures isolated from native species (known to harbor possible sharpshooter vectors) to better define potential sources of inoculum around vineyards for Pierce's disease.

**Justification:** Much of the recent expansion of the winegrape industry in NC is in the Piedmont, a geographic region of the state where production of vinifera/French American hybrid grapes may be at risk for Pierce's disease, caused by the gram-negative bacterium, Xylella fastidiosa (Xf). In surveys conducted in 2001 and 2002 (Harrison and Sutton, unpublished) Xf was detected in grapevines from 14 of 19 vineyards in the NC Piedmont and Mountain regions surveyed in 2001 and 18 of 22 in 2002. In the surveys, vineyards with negative ELISA results were largely limited to areas of the state that have lower average winter temperatures. specifically the mountains of western NC. Xf is vectored by sharpshooters from reservoir hosts to grapevines. In surveys conducted in vineyards in NC in 2002 and 2003 (Bill Hanlin, unpublished), Graphocephala versuta was by far the most abundant potential vector encountered. Xf has a diverse natural host range with over 100 herbaceous and woody plant species, many of which are thought to be symptomless, but may serve as reservoirs of inoculum for insect transmission of the bacterium to grapevines. In our own survey conducted in 2001 and 2002 (Harrison and Sutton, unpublished data), several wild plant species around three NC vineyards with PD-symptomatic vines tested positive for Xf using ELISA test kits, including wild grape, pokeweed, sumac, blackberry, Virginia creeper, and Bermuda grass, and tree species found commonly throughout NC: oak, sycamore, sourwood, sweet gum, wild cherry, beech and hickory. The native species that were found to harbor sharpshooter vectors coincide with many testing positive with ELISA for the Xf bacterium. However, it is not known whether the Xf strains infecting these species (oak, maple, wild cherry, wild grape, blackberry, etc.) will infect grape. Thus the aim of this study was to collect isolates of Xf from common reservoir hosts surrounding the vineyard and test the isolates for pathogenicity to grapes.

**Methodology:** In late summer of 2003, when Pierce's disease symptoms were most visible, attempts were made to collect symptomatic vegetation around vineyards with a history of the disease. Collections focused on hosts that tested positive for *Xf* in 2002, and where the highest populations of sharpshooters, especially *G. versuta*, have been found. Samples were assayed using standard testing procedures (ELISA kits from AgDia, Inc., Elkhart, IN). Isolation procedures were conducted to obtain pure cultures of *Xf* from all hosts.

**Results:** Because of the wet, cool growing season, grapevines were not stressed and symptoms typical of those induced by *Xf* did not appear until mid-September; also PD symptoms were difficult to assess statewide due to severe downy mildew disease. Many of the wild plant species under investigation also appeared symptomless, and results from ELISA test kits during early September were negative. Due to the limited amount of time between symptom expression (mid-September) and the first killing frost (early October), ELISA testing was halted, and samples of native plant hosts that had tested positive with ELISA testing during the previous season (2002) were collected and attempts were made to isolate *Xf* from them. Several different colony types were obtained from these isolations and are still being purified. Once pure, the isolates will be tested with ELISA kits to determine whether they are indeed *Xf*.

**Conclusions:** At this time, we are uncertain as to whether the alternate hosts we sampled were infected with *Xf*. Even though these plants had tested positive for *Xf* in 2002 with ELISA kit testing, it is possible that populations of *Xf* in these same hosts were reduced or eliminated by the more severe winter temperatures in 2003, and as a result the bacterium may not be present in high enough numbers to be successfully isolated.

**Impact Statement:** Vegetation management will be an important tool to minimize the impact of Pierce's disease. However, the relationship between reservoir hosts of *Xf* in NC and potential sharpshooter vectors has not been elucidated in NC, and it is not yet clear which reservoir hosts are important sources of inoculum of *Xf*.

Publications: none